A PARASITE, *MYXOBOLUS HYLAE* Sp. Nov., OF THE REPRODUCTIVE ORGANS OF THE GOLDEN SWAMP FROG, *HYLA AUREA*.

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(Plate XIV.; text figs. 1-5.)

A NUMBER of Golden Swamp Frogs, *Hyla aurea*, which were received from Sydney during 1915 for dissection purposes in this laboratory, were found to have their genitalia infested by a species of *Myxobolus*. This material forms the subject matter of the following paper, though in addition to some preparations made at that time, we have also others made many years previously in Sydney.

The first to record the occurrence of this parasite was A. W. Fletcher, who presented a paper to the first meeting of the Australasian Association for the Advancement of Science in 1888, an abstract of which was published in its Report. Fletcher used the wide term "myxosporidium," but his descriptions leave no doubt that he was dealing with the same organism as we are discussing. The parasite was observed in a considerable proportion of the male frogs dissected in the Biological Laboratory of the University of Sydney, and was observed in one species only, *Hyla aurea*. It was said to have been found also in the urinary bladder under the peritoneum of both males and females, as well as in the testes. Fletcher aptly described the appearance of the infected part as presenting "a large oolitic mass of encysted myxosporidia," and he gave a short account of the spores which he described as closely resembling those of a species occurring encysted on the gills of cyprinoid fish. Several intermediate stages in the development of the spore were observed.

The myxosporidium which Professor W. A. Haswell (1890, p. 661) mentioned as being common in *Hyla aurea* is obviously the same parasite. The only other records of this protozoon were made by one of us (T. H. J., 1909) who referred to *Myxobolus sp.*, a sporozoon infesting and destroying the genital organs of *Hyla aurea* in Sydney, and by Cleland and Johnston (1910) in their paper on the "Haematozoa of Australian Batrachians," where mention was made of its occurrence in that frog in the Sydney district. *H. aurea* does not occur in south-eastern Queensland, but is extremely common in the neighbourhood of Sydney.

The parasite is an interesting one, being the first member of the family Myxobolidæ to be recorde from an amphibian host. Representatives of each of the other families of Myxosporidia are knowd as parasites of Amphibia. In addition, a microsporidian, *Plistophora danilewskyi*, Pfeiffer, has been recorded from the foot-muscles of a European frog, *Rana temporaria*. We append a list of Myxn sporidia known to infest members of the class Amphibia.

Labbé (1899, pp. 87-95) mentions :—

(1) *Leptotheca ohlmacheri* Gurley, as occurring in the kidney and ureters of *Bufo lentiginosus Rana esculenta*, and *R. temporaria*.

(2) *Cystodiscus immersus* Lutz (=*Sphaeromyxa immersa*), in the gall bladder of *Bufo marinus* and *Leptodactylus ocellatus*—both Brazilian batrachians.

(3) *Chloromyxum caudatum* Thélohan, from the gallbladder of *Molge cristata.*

Aurebach (1910, p. 44) refers to the same three species, but omits *Rana esculenta* as a host for *L. ohlmacheri.* He adds a fourth, namely :—

Chloromyxum protei Joseph (1905, 1906), from the kidney of *Proteus anguineus.*

Müller, in 1895, referred to the presence of " Myxosporidia " in tumours in the skin of *Rana temporaria* (see Labbé, 1899, p. 113).

Doflein (1911, pp. 871 and 875) merely mentioned the occurrence of members of Polysporea and, doubtfully, of the Disporea in Amphibia.

Mode of Occurrence.—The infected frogs appear sickly and emaciated. In the male the testes and vasa efferentia are attacked, while in the female only the oviducts have beeu found to harbour the parasite. The disease is much more common among males. In a batch of about thirty *H. aurea,* every male frog (seven) and two females were parasitised (April, 1915). In cases of heavy infection the whole testis is swollen and studded thickly with white cysts, which may be imbedded in the tissue of the organ or may project freely into the cœlome. The largest cysts are 2-3 mm. in diameter, but all sizes, down to those of microscopic dimensions are to be found. Each cyst when crushed exudes a milky fluid, which proves on microscopic examination to be composed of myriads of tiny spores. Small cysts and loose spores may be found in the efferent ducts, but no spores have yet been detected by us in sections of the kidney tubules. Fletcher found the parasite also in the urinary bladder of both sexes. As no fresh material was available for the present work, and as only the genitalia of the diseased specimens were preserved, we are unable to confirm his observation.

Fig. 1.—Transverse section of a heavily infested testis.

Fig. 2.—T.S. infected testis.

In one male specimen both testes and both kidneys were affected, and the upper parts of the ureters adjacent to the kidneys were swollen and milky in appearance. In another, in addition to the testes, the adjacent kidney and mesentery were attacked. In parasitised females, one or both oviducts were infested.

The Spore.—The spore consists of an outer resistant shell or envelope, and an inner protoplasmic body. This envelope is bivalved, the two valves forming a slightly thickened rim where they meet. When lying on its flat surface the spore appears as an oval or sometimes more or less egg-shaped body.

There is a considerable variation in size, but the average dimensions are : Breadth 8-10 μ, thickness about 6 μ. Reduction in length is the commonest variation, some individuals being approximately circular, with a diameter of 7-8 μ. The thickness of the outer shell or envelope is about 1 μ. In some the envelope cell nuclei are visible at the posterior end of the spore. This perhaps represents a developmental stage.

At the anterior end are two prominent pear-shaped polar capsules, the average dimensions of which are 4-5 μ in length and 2 μ in breadth. Each polar capsule contains a fine, spirally-wound polar filament, which is extruded on application of acids or alkalies, and which, when fully extended, measures 90-98 μ in length.

The posterior portion of the spore is occupied by the protoplasmic body—the future sporozoite or amœbula. In the protoplasm there lies a relatively large iodinophilous vacuole, about 2 μ in diameter. The nucleus is usually double ; sometimes there are two distinct nuclei, while rarely only one is detected.

Sections of the kidney, testis, and oviduct were cut, varying in thickness from 1 to 7 μ. In regard to staining methods, Ehrlich's and Delafield's hæmatoxylins gave the best all round results, both for sections and smears, the various nuclei showing up well in some preparations. With Giemsa, differentiation was not so good. Carbol fuchsin acted as a differential stain for the spore ; by overstaining

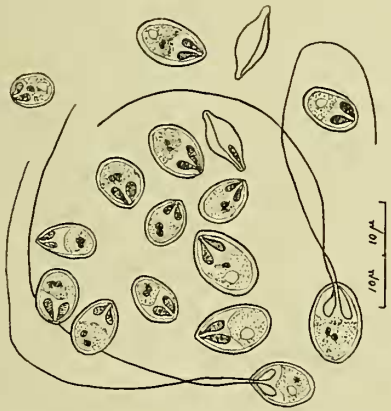

Fig. 3.—Spores of *Myxobolus hylæ*.

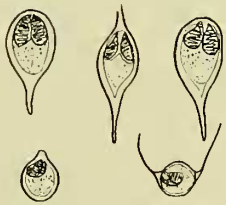

Fig. 4.—Abnormal spores.

and then washing out excess stain in acid alcohol, the host tissue was very faintly tinted while the spores showed up as bright pink. By using borax carmine and thionin the host tissue was stained pink, the spores alone taking the blue dye. The vacuole, polar capsules and threads, as well as the envelope, showed up well on treatment with tincture of iodine. Both Haidenhain's hæmatoxylin and picro-carmine gave unsatisfactory results.

In a section of an infested testis a large portion of that organ may be seen to be occupied by cysts. Each cyst is surrounded by a narrow, lightly-staining ring—the ectoplasm. Immediately within this is a denser, more or less granular layer containing developing spores, while within this again the cyst is closely packed with fully-developed spores. In the very small cysts few or no mature spores are distinguished. In sections stained with carbol fuchsin masses of spores may be noticed lying in the seminiferous tubules along with the sperms.

In a section of an infected oviduct, the cyst was observed to lie between the layers of the wall and to project into the lumen of the duct. It possessed the same structure as described above.

Stages in Development.—Cells with a single nucleus were fairly common in smears made from a cyst, and perhaps represented the young pansporoblasts. Cells with 2, 4, 6 and 14 nuclei were seen, and were probably stages in the development of the spores within the pansporoblast. Other larger cells, with prominent irregular nuclei and smaller, more deeply-staining chromatin masses in the cytoplasm, were also met with.

Fig. 5.—Some developmental stages: *a*, amœbula; *e c n*, envelope cell nucleus; *p c*, developing polar capsule; *p c c*, polar capsule cell.

Experimental.—In April, 1915, two infected testes were removed from a Golden Swamp Frog, *Hyla aurea*, and fed to two Green Tree Frogs, *H. cærulea*. After three weeks, one was killed, but an examination of it failed to reveal the presence of *Myxobolus*; the other frog escaped. In November, 1915, cysts from a female *H. aurea* were fed to another *H. cærulea*. After four weeks the latter seemed sickly and was killed, but no myxosporidia were detected within it.

The parasite is apparently specific in its associations, since it has not been observed in any frog other than *H. aurea*. Both *H. aurea* and *H. cærulea* occur around Sydney; the former abounds in swamps, and is much more common than its larger tree-climbing relative.

It has already been pointed out by Cleland and Johnston that the latter harbours a blood parasite, *Lankesterella hylæ*, which has never been detected in the Golden Swamp Frog.

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EXPLANATION OF PLATE XIV.

A and B.—T.S infected testes × 30. In A the sporozoon cysts are situated towards the inner border of the gland, some actually projecting into an efferent duct.

C.—Portion of section figured in B, showing two cysts with abundant spores, also numerous clusters of spores in the ducts of the testis. × 68

D.—Portion of the large cyst shown in B. Edge of cyst showing (*a*) mature spores, (*b*) granular zone, containing developing spores, (*c*) narrow ectoplasmic zone, (*d*) tissue of testis. × 200.

E.—Part of oblique section of oviduct. Lettering as in D; also (*e*) layer of large cells belonging to oviduct, (*f*) epithelium, and (*g*) lumen of duct. × 400.